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LEAF RUST RESISTANCE IN WINTER BARLEY CULTIVARS AND BREEDING LINES

ABSTRACT

Leaf rust is economically important disease of barley in many barley growing countries including Poland. A total of 25 winter barley cultivars and breeding lines were tested for leaf rust resistance with eight differential isolates. These isolates originated from IHAR Radzików collection and were chosen according to their virulence spectra. Among 25 cultivars and breeding lines only 7 (28%) showed resistance reaction after inoculation with at least one isolate of *P. hordei*. In only one cultivar Kroton it was possible to postulate the presence of specific resistance genes which were *Rph2* and *Rph6*. Eighteen (72%) cultivars and breeding lines showed susceptible reaction after inoculation with all isolates used. Based on results, we can conclude that these cultivars have no resistance gene to *P. hordei* or they may have one or combination of three resistance genes (*Rph1*, *Rph10*, *Rph11*). Among tested cultivars and breeding lines the most resistant was breeding line POA 2099. It showed resistance for inoculation with 3 isolates of *P. hordei*. Only 5.0% of infection types observed on plants of tested cultivars and breeding lines were classified as leaf rust resistance [scores 0 (2%) and 2 (3%)]. None of tested cultivars and breeding lines showed resistance reaction types 0; and 1. Different strategies for control of barley leaf rust were discussed.

Key words: barley, cultivar, leaf rust, *Puccinia hordei*, resistance

INTRODUCTION

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop worldwide. Leaf rust, caused by the fungal pathogen *Puccinia hordei* Otth, is an important foliar disease on barley in most regions throughout the world including Europe (Clifford 1985), North America (Griffey *et al.* 1994, Roane 1962, Sharp and Reinhold 1982), Near East (Anikster 1982, 1984, Anikster *et al.* 1992, Brodny *et al.* 1992), New Zeland (Lim and Gaunt 1986, Teng *et al.* 1979), Australia (Park *et al.* 1992) and North Africa (Parlevliet *et al.* 1981, Yahyaoui and Sharp 1987). In some regions of Poland this disease may cause economically significant losses (Mazaraki and Grabowska 1998). Generally, in Central Europe leaf rust ranks second after powdery mildew among the most common diseases of barley (Czembor *et al.* 2006, Dreiseitl and Jurecka 1996, 1997). Recently the breeders interest in resistance to barley leaf rust has in-

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creased in Europe (Mazaraki and Grabowska 1998; Niks *et al.* 2000; Czembor *et al.* 2006; Czembor and Bladenopoulos 2007, Czembor and Czembor 2007a). This interest is caused by observations of increases in fitness of leaf rust populations to many currently grown barley cultivars and to cultivar Vada. Cultivar Vada is well known for possessing high level of partial resistance to leaf rust and it was widly used as parent in major European barley breeding programs (Czembor and Czembor 2007a, Rients Niks personal communications).

Barley yield losses may reach 30% in susceptible cultivars due to infection by *P. hordei* (Griffey *et al.* 1994, Whelan *et al.* 1997). However the average yield losses of barley due to leaf rust reach usually 10-25% (Niks *et al.* 2000). It has to be stressed that often more important than lowering of barley yield by leaf rust is loss of its quality. This lost of quality of grain especially concerns plantations of barley for malting purpose Griffey *et al.* 1994, Niks *et al.* 2000). Growing of barley cultivars possessing resistance to leaf rust has been efficient mean for preventing yield losses due to infection by this pathogen. This strategy of leaf rust control still can be successfully used in barley breeding programmes because 19 loci with major genes for resistance to leaf rust are described: *Rph1*, *Rph2bj*, *k*, *l*, *m*, *n*, *q*, *r*, *s*, *t*, *u*, *y*, *Rph3c*, *w*, *aa*, *Rph4*, *Rph5*, *Rph6*, *Rph7g*, *ac*, *Rph8*, *Rph9*, *Rph10*, *Rph11*, *Rph12*, *Rph13*, *Rph14*, *Rph15*, *Rph16*, *Rph17*, *Rph18*, *Rph19*) (Chełkowski *et al.* 2003, Franckowiak 2002, Franckowiak *et al.* 1997, Park and Karakousis 2002, Park *et al.* 2003). In addition, barley geneticists and phytopathologists are describing new leaf rust resistance loci. Barley breeders can use these newly described genes in their breeding programmes (Alemayehu and Parlevliet 1996, Manisterski and Anikster 1995, Czembor 2007a, 2007b, Czembor and Czembor 2007b).

In order to proper use new sources of resistance to leaf rust barley breeders and phytopathologists have to know which virulences are most frequent in Europe (Mazaraki and Grabowska 1998; Niks *et al.*, 2000). In addition the resistance alleles present in cultivars and breeding lines used in agriculture have to be known in order to interpret and predict interactions between populations of the *P. hordei* and barley cultivars. Therefore, tests of the cultivars and breeding lines had to be carried out for identifying alleles for leaf rust resistance. This identification is conducted on the basis of the gene-for-gene hypothesis. Using this hypothesis it is possible to identify such genes by inoculation of plants with pathogen isolates that have a defined, well-known virulence spectrum and the subsequent reading of infection types. This method is commonly used in breeding programmes of barley for resistance to infection by obligate pathogens such as rusts and powdery mildews (Czembor 1996, 2005, Dreiseitl and Steffenson 2000, Czembor and Bladenopoulos 2007, Czembor and Czembor 2007a).

The aim of the presented investigation was to identify the leaf rust resistance genes in winter barley cultivars and breeding lines included in Polish official trials.

MATERIALS AND METHODS

Plant material

A total of 25 barley cultivars and breeding lines from Polish register were tested (Table 1). Seed samples of these cultivars were kindly provided by their breeders.

Table 1

Twenty five cultivars and breeding lines of winter barley with their country of origin, status, breeder and year of entry of Polish Cultivar Register (Anonymous 2001)

Cultivar	Country of origin	Status of a cultivar	Year of entry in the Register	Breeder
Kos	PL	R	1989	ZDHAR Bąków
Gil	PL	R	1990	ZDHAR Bąków
Sigra	DE	R	1990	Lochow - Petkus GmbH
Marinka	NL	R	1991	Cebeco Zaden B.V.
Kroton	PL	R	1992	SHR Modzurów
Gregor	PL	R	1993	SHR Modzurów
Horus	PL	R	1996	SHR Marchwacz
Borwina	DE	Re	1998	I.G. Saatzucht GmbH & Co. KG
BKH 2198	PL	T	1998	ZDHAR Bąków
LP 6-562	DE	T	1998	Lochow-Petkus
Tramp	PL/DE	R	1998	SHR Modzurów
Paweł	PL	Re	1999	"Grupa Danko" - Sobiejuchy
Tiffany	DE	R	1999	Saatzucht Josef Breun GdbR
Carola	DE	T	1999	Nordsaat
CWB 96-9	UK	T	1999	PBI Monsanto
LP 6-758	DE	T	1999	Lochow-Petkus
POA 2099	PL	T	1999	Piast HR Łagiewniki
BKH 2399	PL	T	1999	HR Smolice
Hamu	DK	T	1999	Sejet PlantBreeding
BKH 2400	PL	T	2000	HR Smolice
CWB 98-103	UK	T	2000	PBI Monsanto
GW 2015	DE	T	2000	Nordsaat Saatzucht
GW 2016	DE	T	2000	Nordsaat Saatzucht
POA 2100	PL	T	2000	SHR Modzurów
Bombay	DE	R	2001	Saatzucht Josef Breun GdbR

R - Original cultivar entered in the National List
T- Original cultivar or breeding line entered to the official trials
Re - Original cultivar removed from the National List

Pathogen

Eight differential isolates of *P. hordei* were used (Table 2). These isolates originated from IHAR Radzikow collection and were chosen according to differences in virulence spectra observed on 12 differential cultivars. None of the isolates used was able to differentiate genes *Rph4* from *Rph8* and *Rph1* from *Rph10* and *Rph11*.

Table 2

Differential isolates used and their infection types

Accession name	Accesion number	Gene	Isolates								References
			Ph- 9	*Ph-5*	*Ph-4*	*Ph-6*	*Ph-31*	*Ph-21*	*Ph-17*	*Ph-25*	
Sudan	CIho 6489	*Rph1*	4	4	4	4	4	4	4	4	Roane and Starling (1967)
Peruwian	CI 935	*Rph2*	4	4	4	4	4	4	2	4	Levine and Cherewick, 1952; Starling, 1956
Estate	CI 3410	*Rph3*	0	4	0	4	4	0	4	4	Henderson, 1945; Roane and Starling, 1967
Gold	CI 1145	*Rph4*	4	4	0	4	4	4	4	4	Moseman and Reid, 1961; Roane, 1962; Roane and Starling, 1967
Magnif	CI 13860	*Rph2+ Rph5*	4	1	4	0	0	0	1	4	Frecha, 1970; Yahyaoui and Sharp, 1987
Bolivia	CI 1257	*Rph2+ Rph6*	4	4	4	4	0	4	4	4	Henderson, 1945; Starling, 1956; Roane and Starling, 1967, 1970
Cebada Capa	CI 6193	*Rph7*	0;	0;	0;	0;	0;	0;	0;	0;	Johnson, 1968; Starling, 1956; Nover and Lehman, 1974; Parlevliet, 1976
Egypt 4	CI 6481	*Rph8*	4	4	0	4	4	4	4	4	Levine and Cherewick, 1952; Tan, 1977
HOR 2596	CI 1243	*Rph9*	4	4	4	4	4	1	4	4	Tan, 1977
Cliper C8	None	*Rph10*	4	4	4	4	4	4	4	4	Feuerstein *et al*., 1990
Cliper C67	None	*Rph11*	4	4	4	4	4	4	4	4	Feuerstein *et al*., 1990
Triumph	PI 290195	*Rph12*	4	4	4	4	4	0;	4	4	Walther, 1987; Jin *et al*. 1993

Testing procedure

This study was carried out in the IHAR Radzikow greenhouse. Cultivar L94 was used as a susceptible control it does not carry any minor and major genes to *P. hordei.* The plants were grown with 16h light and temperature range of 18-22^{o}C. Urediniospores of *P. hordei* were suspended in deionized water with couple drops of mineral oil “Twin 20" and inoculated onto one week old seedling plants (primary leaf fully expanded) using a rate 3 mg urediniospores × 10 ml of water^{-1} × 100 plants $^{-1}$. Inoculated plants were incubated for 24 hours in a chamber in which the humidity was maintained near saturation by mist from ultrasonic humidifiers. Also, during this 24 hours, plants were kept in complete darkness and in temperature range of 12-15°C. Then plants were transferred to a greenhouse bench.

Disease assessment

Reactions of each accession were evaluated after an incubation period of 12-14 days in a greenhouse at 20-24°C range of temperature. Disease symptoms were assessed on the primary leaf of the seedlings according to 0-4 scale adapted from Levine and Cherewick (1952) (Table 3). Infection types 0, 0;, 1 and 2 were considered indicative of host resistance and infection types 3 and 4 of host susceptibility.

Table 3

Description of infection types and codes used (adapted from Levine and Cherewick 1952).

Infection Type	Host Response	Symptoms
0	Immune	No vivible uredia
0;	Very resistant	Hypersensitive flecks
1	Resistant	Small uredia with necrosis
2	Moderately resistant	Small to medium sized uredia with green islands and surrounded by necrosis or chlorosis
3	Moderately susceptible	Medium sized uredia with or without chlorosis
4	Susceptible	Large uredia without chlorosis

Postulation of leaf-rust resistance genes

Accessions exhibiting the same reaction pattern as a specific differential line were postulated to carry the respective *Rph* gene. It was made on the basis of gene-for-gene hypothesis.

RESULTS

Among 25 cultivars and breeding lines tested only 7 (28%) showed resistance reaction after inoculation with at least one isolate of *P. hordei* (Table 4). Eighteen (72%) cultivars and breeding lines showed susceptible reaction after inoculation with all isolates used. These cultivars have no resistance gene to *P. hordei* or they may have one or combination of three resistance genes (*Rph1*, *Rph10*, *Rph11*).

Among tested cultivars and breeding lines the most resistant was line POA 2099. It showed resistance for inoculation with 3 isolates of *P. hordei*. In only one cultivar Kroton it was possible to postulate the presence of specific resistance genes *Rph2* and *Rph6*.

Only 5.0% of infection types observed on plants of tested cultivars and breeding lines were classified as leaf rust resistance [scores 0 (2%) and 2 (3%)]. None of tested cultivars and breeding lines showed resistance reaction types 0; and 1.

Table 4

Reaction of 25 cultivars and breeding lines of spring barley to infection by eight isolates of *Puccinia hordei*.

Cultivar	Isolate								Postulated resistance alleles	Possible alleles
	Ph-9	Ph-5	Ph-4	Ph-6	Ph-31	Ph-21	Ph-17	Ph-25		
Kos	4	4	4	4	4	4	4	4	?	*[Rph1, Rph10, Rph11]*
Gil	4	2	4	4	4	4	4	4	?	
Sigra	4	4	4	4	4	4	4	4	?	*[Rph1, Rph10, Rph11]*
Marinka	4	4	4	4	4	4	4	4	?	*[Rph1, Rph10, Rph11]*
Kroton	4	4	4	4	0	4	4	4	*Rph2, Rph6*	
Gregor	4	4	4	4	4	4	4	4	?	*[Rph1, Rph10, Rph11]*
Horus	4	4	4	4	4	4	4	4	?	*[Rph1, Rph10, Rph11]*
Borwina	4	4	4	4	4	4	4	4	?	*[Rph1, Rph10, Rph11]*
BKH 2198	3	4	4	4	4	4	4	4	?	
LP 6-562	4	2	4	4	4	4	4	4	?	
Tramp	4	4	4	4	4	4	4	4	?	*[Rph1, Rph10, Rph11]*
Paweł	4	4	4	4	4	4	4	4	?	*[Rph1, Rph10, Rph11]*
Tiffany	4	4	0	4	0	4	4	4	?	*[Rph2, Rph6, Rph4, R[ph8]*
Carola	3	4	4	4	4	4	4	4	?	*[Rph1, Rph10, Rph11]*
CWB 96-9	4	4	4	2	4	4	4	4	?	
LP 6-758	4	4	4	4	4	4	4	4	?	*[Rph1, Rph10, Rph11]*
POA 2099	2	2	4	4	4	4	2	4	?	
BKH 2399	4	4	4	4	4	4	4	4	?	*[Rph1, Rph10, Rph11]*
Hamu	4	4	4	4	4	4	4	4	?	*[Rph1, Rph10, Rph11]*
BKH 2400	4	4	4	4	4	4	4	4	?	*[Rph1, Rph10, Rph11]*
CWB 98-103	4	4	4	4	4	4	4	4	?	*[Rph1, Rph10, Rph11]*
GW 2015	4	0	4	4	4	4	4	4	?	
GW 2016	4	4	4	4	4	4	4	4	?	*[Rph1, Rph10, Rph11]*
POA 2100	4	4	4	4	4	4	4	4	?	*[Rph1, Rph10, Rph11]*
Bombay	4	4	4	4	4	4	4	4	?	*[Rph1, Rph10, Rph11]*

DISCUSSION

Many studies showed that *P. hordei* is characterised by large genetic variability (Brodny and Rivadeneira 1996, Fetch *et al.* 1998, Park 2003). Introduction of specific resistance genes in barley cultivars quickly results in selection of virulent races of *P. hordei*. One of exaples for this is deployment in barley cultivars of gene *Rph7* originating from Cebada Capa. This gene was the most effective leaf rust resistance genes in barley. Cultivars with this gene were widely grown in the southeastern US beginning in the late 1960s. It remained

effective until the early 1990s. At this time virulence was detected in collections from the southeast of US and California (Steffenson *et al.* 1993). Virulence to *Rph7* occurred previously in Israel and North Africa. However, the origin of this virulence in the US was considered most likely to be due to mutation and selection (Alemayehu 1995, Niks *et al.* 2000, Steffenson *et al.* 1993).

In Poland and other countries of Europe, farmers apply repeated fungicide treatments on barley to protect against fungal leaf pathogens, including *P. hordei*. However, there is increasing opposition in societies of these countries to the application of large amounts of pesticides in agriculture. Main reason for this is concern on environmental and health risks (Czembor 2005, Nieróbca *et al.* 2003). The obvious alternative to fungicide treatment against plant diseases is the use of resistant cultivars (Alemayehu 1995, Cotterill *et al.* 1995, Czembor 2005, Dreiseitl and Steffenson 2000). The obtained results indicated lack of resistance or very low level of resistance to *P. hordei* in barley cultivars and breeding lines. Considering this fact, it is recommended to use fungicides to control barley leaf rust and to use resistance genes in various strategies for resistance gene deployment (Czembor 1996, 2005, Finckh *et al.* 2000). In addition there is need to identify and use new sources of resistance to this pathogen in barley breeding programes (Alemayehu and Parlevliet 1996, Manisterski and Anikster 1995, Czembor 2007a, 2007b, Czembor and Czembor 2007b).

Obtained results showed that tested cultivars and breeding lines of winter barley is significantly lower than it was reported for breeding lines and cultivars of spring barley (Czembor and Czembor 2007a). Among 25 cultivars and breeding lines tested only 7 (28%) showed resistance reaction after inoculation with at least one isolate of *P. hordei*. Eighteen (72%) cultivars and breeding lines showed susceptible reaction after inoculation with all isolates used. These cultivars have no resistance gene to *P. hordei* or they may have one or combination of three resistance genes (*Rph1*, *Rph10*, *Rph11*). Only 5.0% of infection types observed on plants of tested cultivars and breeding lines were classified as leaf rust resistance [scores 0 (2%) and 2 (3%)]. Among tested cultivars and breeding lines the most resistant was line POA 2099. It showed resistance for inoculation with 3 isolates of *P. hordei*. In only one cultivar Kroton it was possible to postulate the presence of specific resistance genes *Rph2* and *Rph6*.

Presented investigations were carried out on barley seedlings and are sufficient for postulation of presence of resistance genes for breeders need (Dreiseitl and Steffenson 2000, Czembor and Bladenopoulos 2007, Czembor and Czembor 2007a). Further studies are needed to determine the number of genes, the types of gene, the type of gene action and the gene loci in resistant lines and cultivars. It may be established only by crosses and backcrosses among appropriate genotypes (Alemayehu 1995, Jin and Steffenson 1994, Martinez *et al.* 2001). Many studies showed that partial resistance is generally better expressed in the adult plant stage (Parlevliet and van Ommeren 1975,

Smit and Parlevliet 1990). Based on this it will be interesting to extend presented studies to adult plants as well.

Described investigation resulted in information about resistance alleles for leaf rust present in cultivars and breeding lines. This kind of information will help breeders to use proper breeding initial material and to use the most effective breeding techniques in breeding barley resistant to this pathogen (Czembor 2005, Vallavieille-Pope *et al.* 2000). Results obtained in described investigation provide information which is crutial for proper interpretation of interactions between populations of the *P. hordei* and barley. Based on this analysis barley breeders can apply the most effective methods for deployment of available resistance genes in grown cultivars.

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